Composition of the leaf volatile terpenoids of *Pinus mugo* Turra from Bulgaria compared with oils from other regions.

Robert P. Adams
Biology Department, Baylor University, Waco, TX 76798 robert_adams@baylor.edu

and

Alexander N. Tashev
University of Forestry, Dept. of Dendrology
10, Kliment Ochridsky Blvd., 1797 Sofia, Bulgaria

ABSTRACT

The volatile leaf oil of *Pinus mugo,* Bulgaria, is composed of 66 components (with numerous additional trace compounds) with large amounts of δ-3-carene (24.6%), β-phellandrene (16.7%), α-pinene (11.0%), and lesser concentrations of β-pinene (2.7), terpinolene (3.9), bornyl acetate (4.2), (E)-caryophyllene (5.3), germacrene D (1.7), α-cadinol (1.1), palustral (1.0). The leaf volatile oil of *P. mugo* from Bulgaria is quite similar to oils from other Balkan populations as per the literature. However, an analysis from *P. mugo* oil from central Italy was quite different from *P. mugo* from the Balkans, indicating the need for additional research on *P. uncinata* leaf oils. Published on-line www.phytologia.org *Phytologia 101(1): 74-80 (March 21, 2019).* ISSN 030319430.

KEY WORDS: *Pinus mugo* subsp. *mugo,* Bulgaria, volatile leaf oil, terpenes, composition, *Pinus mugo subsp. uncinata* (*P. uncinata*).

Pinus mugo Turra has been treated as composed of two subspecies: subsp. *mugo* and subsp. *uncinata* (Raymond) Domin (Chrsitensen 1987) or as two species: *P. mugo* and *P. uncinata* (Richardson 1998). *Pinus mugo* subsp. *mugo* is distributed in northern Italy into the Balkans and in eastern Europe and *P. uncinata* is distributed from the Alps, southwestward into France and Spain with hybridization between the subspecies in the zone of contact in the central European alps (Richardson, 1998).

The volatile leaf essential oils (terpenes) of *P. mugo* have been analyzed from Italy (Bambagiotii and Vincieri 1972; Venditti et al. 2013, Table 1), Macedonia (Karapandzova et al. 2011; Slovenia (Bojovic et al. 2016, Table 1); Sar Mountains (Mitic et al. 2017, Table 1) and cultivated, Cambridge Botanic Gardens (Ioannou et al. 2014). The volatile oil of P. uncinata (*P. mugo* subsp. *uncinata*) has been reported from central Italy (Venditti, et al. 2013) and from cultivated trees in Poland (Celinski et al. 2015, Bonikowski et al. 2015).

Several other papers cited *P. mugo* volatile analyses, but samples were obtained from cultivated materials of unknown source, and thus, not of interest in this study of geographical variation in *P. mugo* subsp. *mugo*.

MATERIALS AND METHODS

Leaf samples collected: *Pinus mugo* subsp. *mugo*, common at edge of a meadow, near forest by *Pinus peuce* and *P. heldrichii* with *Juniperus communis*. Bulgaria, 47° 45' 52.8" N, 23° 25' 22.6" E., 1838 m. Bulgaria, Coll. *Alex Tashev 1-5*, 12 June 2018, Lab Acc. *Robert P. Adams 15495, 15496, 15497, 15498, 15499*. Voucher specimens are deposited in the herbarium, Baylor University.

Gently dried leaves (100g, 40 - 45˚C) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Note that limonene and β-phellandrene elute as a single peak on DB-5, but their amounts can be quantitated by the ratio of masses 68, 79 (limonene) and 77, 93 (β-phellandrene). Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The composition of the volatile leaf oil of *Pinus mugo* subsp. *mugo* from Bulgaria is given in table 1. The volatile leaf oil contains large amounts of δ-3-carene (24.6%), β-phellandrene (16.7%), and α-pinene (11.0%), with moderate concentrations of β-pinene (2.7), terpinolene (3.9), bornyl acetate (4.2), (E)-caryophyllene (5.3), germacrene D (1.7), α-cadinol (1.1) and palustral (1.0). This compositional pattern seems common in the Balkan populations (Slovenia, Kosovo, Table 1). However, the putative *P. mugo* subsp. *mugo* from central Italy (Table 1, Venditti et al. 2013) has a very different terpene profile with δ-3-carene (0.8%), β-phellandrene (1.2%), and α-pinene (trace) being very small, as well as numerous other components being different (Table 1). This suggests the central Italy population may be *P. uncinata* (*P. mugo* subsp. *uncinata*). Recently, Boratynska et al. (Fig. 1, 2015) reported the natural ranges of *P. mugo* and *P. uncinata*, but subsequent morphological analyses revealed the central Italy *P. mugo* population to be *P. uncinata* (Fig. 5, Boratynska et al. 2015). Celinski et al. (2015), using head-space to analyze the oil of *P. uncinata* (cultivated in Poland, natural source not reported), so their results are not exactly compatible with steam distilled oils compositions (Table 1). However, coupled with the morphological analysis of Boratynska et al. (2015) that indicates the central Italy population is most like *P. uncinata*, it may be that the central Italy oil (Table 1) is that of *P. uncinata.* Additional research is needed to resolve this.

Variation among the individuals in the Bulgaria, *P. mugo* population (Table 2) is moderate with ranges of: δ-3-carene (19.8 - 28.2%), β-phellandrene (12.8 - 21.6%), α-pinene (7.3 - 17.1 %), β-pinene (1.7 - 3.3), terpinolene (3.1 - 4.6), bornyl acetate (1.6 - 8.9), (E)-caryophyllene (4.2 - 6.8), germacrene D (0.4 - 2.9), germacrene D-4-ol (1.2 - 3.3) and palustral (0.6 - 1.5). No chemotypes were apparent.

In conclusion, the leaf volatile oils of *P. mugo* from Bulgaria were quite similar to those from other Balkan populations reported in the literature (Table 1).

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Table 1. The leaf oil constituents of *Pinus mugo* from Bulgaria compared with other analyses on the volatile leaf oils. Compounds in bold face vary among locations. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear calculation on DB-5 column. Note: *P. uncinata* analysis was based on head space volatiles (Celinski, et al. 2015), so it is not exactly compatible with the other steam distilled oils' compositions.

KI	compound	Bulgaria *15773* this study	Kosovo Oshlak Hajdari[1]	Kosovo Hajle Hajdari[1]	Sar Mtns. s. Kosovo Mitic[2]	Slovenia Julian Alps Bojovic[3]	Cambridge UK, Cult. Iannou[4]	*uncinata* central Italy Venditti[5]	*uncinata* cultivated Poland Celinski[6]
921	tricyclene	0.2	0.9	0.9	0.8	0.4 – 0.6	0.5	-	0.8
924	α-thujene	0.3	1.3	t	1.5	0.5 – 0.8	1.2	-	-
932	**α-pinene**	**11.0**	**17.0**	**19.9**	**18.0**	**12.9 -17.6**	**13.7**	**t**	**27.8**
946	**camphene**	**1.2**	**3.2**	**3.2**	**3.0**	**1.5 – 2.2**	**2.4**	**-**	**9.9**
969	sabinene	1.2	1.0	0.9	1.0	1.3 – 1.6	1.1	-	-
974	**β-pinene**	**2.7**	**5.5**	**2.8**	**4.1**	**2.7 - 8.7**	**2.1**	**0.1**	**-**
988	myrcene	3.1	2.6	2.4	2.2	2.9 – 12.2	6.9	0.1	8.9
1002	α-phellandrene	0.5	0.5	1.0	0.4	0.2 -0.6	-	t	0.2
1008	**δ-3-carene**	**24.6**	**17.7**	**27.9**	**21.3**	**13.0 – 27.0**	**9.9**	**0.8**	**t**
1014	α-terpinene	0.2	0.3	0.2	0.6	0.2 – 0.2	0.5	t	0.1
1020	p-cymene	0.2	0.2	0.3	0.1	0.1 – 0.2	-	t	-
1024	limonene	t	t?	t?	t?	t	t	t	7.0
1025	**β-phellandrene**	**16.7**	**5.9**	**3.8**	**7.6**	**14.5 – 18.0**	**2.6**	**1.2**	**7.6**
1044	(E)-β-ocimene	0.6	1.2	0.8	0.5	t	0.6	0.3	6.4
1054	γ-terpinene	0.4	0.5	0.4	0.7	0.3 – 0.5	0.4	0.1	0.2
1086	**terpinolene**	**3.9**	**4.3**	**2.8**	**5.5**	**3.0 - 3.4**	**3.8**	**0.5**	**0.8**
1095	linalool	-	0.2	t	0.2	-	-	0.3	-
1118	cis-p-menth-2-en-1-ol	0.1	-	-	-	t	-	0.3	-
1132	cis-limonene oxide	0.1	-	-	-	-	-	0.5	-
1136	trans-sabinol	0.1	-	-	-	-	-	-	-
1141	camphor	-	0.2	0.5	t	-	-	0.2	-
1145	camphene hydrate	-	-	-	t	-	-	0.3	-
1165	borneol	0.2	0.5	0.3	0.2	t	-	1.5	-
1174	**terpinen-4-ol**	**0.4**	**t**	**t**	**0.9**	**0.2 – 0.3**	**0.2**	**3.6**	**-**
1176	m-cymen-8-ol	0.2	t	t		-	-	0.5	-
1179	p-cymen-8-ol	0.3	t	t	t	-	-	1.6	-
1183	cryptone	-	-	-	-	t	-	0.4	-
1186	**α-terpineol**	**t**	**0.2**	**t**	**0.4**	**t**	**-**	**7.3**	**t**
1195	myrtenol	0.2	0.2	0.2	t	-	-	0.2	-
1204	verbenone	0.1	-	-	-	t	-	-	-
1207	trans-piperitol	-	-	-	-	-	-	0.4	-
1223	citronellol	t	-	-	-	-	-	0.2	-
1226	cis-carveol	-	-	-	-	-	-	0.3	-
1232	thymol, methyl ether	0.5	0.3	t	0.2	0.3 – 0.5	-	0.7	-
1249	piperitone	-	-	-	-	-	-	1.0	-
1253	trans-sabinyl acetate	0.2	-	-	-	-	-	-	-
1254	linalool acetate	0.2	0.2	0.3	0.2	-	-		-
1274	pregeijerene B	-	-	-	-	-	-	0.6	-
1284	**bornyl acetate**	**4.2**	**4.3**	**4.6**	**5.1**	**2.3 – 3.5**	**3.8**	**11.5**	**10.4**
1293	2-undecanone	0.3			0.1	t	-	0.2	-
1315	(2E,4E)-decadienal	0.1					-		-
1345	α-terpinyl acetate	1.8			1.4	0.8 – 1.0	-	2.4	t
1389	β-elemene	0.2	1.7	0.7	0.6	0.7 – 1.6	1.2	0.3	t
1417	**(E)-caryophyllene**	**5.3**	**5.3**	**4.5**	**5.0**	**4.8 – 6.0**	**5.3**	**5.9**	**5.1**
1439	aromadendrene	-	0.3	0.2	-	-	-	-	-
1442	6,9-guaiadiene	-	-	-	-	-	-	1.4	-
1454	α-humulene	0.8	0.6	0.5	0.8	0.8 – 1.0	-	1.2	1.0
1454	(E)-β-farnesene	0.2	-	-	-	-	-	0.1	-
1477	β-chamigrene	-	0.4	0.2	-	-	-	-	-
1478	γ-muurolene	0.2	0.4	0.2	-	t	-	-	0.2
1480	**germacrene D**	**1.7**	**9.9**	**4.0**	**5.6**	**3.9 -5.0**	**12.1**	**0.6**	**0.9**
1489	β-selinene	-	0.2	0.2	-	t	-	-	-

KI	compound	Bulgaria *15773* this study	Kosovo Oshlak Hajdari[1]	Kosovo Hajle Hajdari[1]	Sar Mtns. s. Kosovo Mitic[2]	Slovenia Julian Alps Bojovic[3]	Cambridge UK, Cult. Iannou[4]	*uncinata* central Italy Venditti[5]	*uncinata* cultivated Poland Celinski[6]
1500	bicyclogermacrene	1.1	2.2	3.4	1.7	1.4 – 2.7	-	0.3	1.1
1500	α-muurolene	0.3	0.6	0.3	0.6	t	-	0.3	-
1508	germacrene A	0.4	-	-	0.2	-	-		-
1513	γ-cadinene	0.6	0.7	0.6	0.8	0.3 – 0.8	-	0.3	0.4
1522	δ-cadinene	1.2	2.3	2.2	2.6	1.0 – 2.1	-	1.2	1.0
1537	α-cadinene	0.1	0.5	0.2	0.2	t	-	0.1	t
1561	(E)-nerolidol	0.1	0.6	0.7		-	-	0.1	-
1574	germacrene-D-4-ol	2.3	-	-	0.2	0.7 – 1.9	-		t
1577	spathulenol	-	1.1	1.3		-	-	0.8	-
1583	caryophyllene oxide	0.4	1.5	1.8	0.1	0.2 – 0.2	-	2.0	-
1608	humulene epoxide II	-	-	-	-	-	-	0.4	-
1638	epi-α-cadinol	0.4	0.7	0.7	-	-	-	1.3	-
1640	epi-α-muurolol	0.4	-	-	0.7	-	-	1.3	-
1644	α-muurolol	0.1	t	t	0.1	-	-	0.5	-
1652	**α-cadinol**	**1.1**	-	-	**0.9**	**0.4 – 0.8**	**2.1**	**4.1**	-
1710	pentadecanal	0.2	-	-	-	-	-	-	-
1880	(3Z)-hexenyl cinnama	0.5	-	-	-	-	-	-	-
1933	cyclohexadecanolide	t	-	-	-	-	-	-	-
1943	iso-cembrene	0.1	-	-	-	-	-	-	-
1987	manool oxide	0.2	0.2	0.2	0.1	t	-	1.0	-
2010	**13-epi-manool oxide**	-	-	-	-	**t**	-	**2.9**	-
2056	abietatriene	0.1	-	-	-	-	-	0.7	-
2087	abietadiene	0.1	t	t	-	-	-	0.5	-
2105	iso-abienol	0.2	-	-	-	-	-	-	-
2149	abienol	t	-	-	-	-	-	1.6	-
2153	abieta-(8(14,13j(15)-d	t	-	-	-	-	-	0.6	-
2243	**palustral (8,13-abietadien-18-al**	**1.0**	-	-	-	-	-	**8.7**	-
2274	**dehydro abietal**	**0.1**	-	-	-	-	-	**4.3**	-
2313	abietal	t	-	t	-	-	-	0.8	-

[1]Hajdari et al. 2015; [2]Mitic et al. 2017; [3]Bojovic et al. 2016; [4]Ioannou et al. 2014; [5]Venditti et al. 2013; [6]Celinski, et al. 2015.

Table 2. Variation in constituents of the leaf volatile oil of *P. mugo* subsp. *mugo* in a population in Bulgaria.

KI	compound	*15495*	*15496*	*15497*	*15498*	*15499*
921	tricyclene	0.2	0.7	0.2	0.2	0.3
924	α-thujene	0.1	t	0.7	0.1	0.3
932	**α-pinene**	**17.1**	**11.0**	**15.1**	**7.3**	**9.5**
946	camphene	1.0	2.5	1.1	1.0	1.2
969	sabinene	1.2	1.2	1.3	1.1	1.2
974	**β-pinene**	**3.3**	**3.0**	**3.1**	**1.7**	**3.0**
988	**myrcene**	**3.3**	**3.3**	**3.4**	**2.4**	**3.1**
1002	α-phellandrene	0.7	0.3	0.6	0.3	0.3
1008	**δ-3-carene**	**26.3**	**23.1**	**19.8**	**28.8**	**27.2**
1014	α-terpinene	0.2	0.2	0.3	0.2	0.2
1020	p-cymene	0.2	0.2	0.1	0.3	0.3
1024	limonene	t	t	t	t	t
1025	**β-phellandrene**	**12.8**	**13.8**	**21.6**	**16.6**	**18.8**
1044	(E)-β-ocimene	0.8	0.6	0.3	0.6	0.8
1054	γ-terpinene	0.3	0.4	0.4	0.4	0.4
1086	**terpinolene**	**3.6**	**3.4**	**4.6**	**3.1**	**3.9**
1118	cis-p-menth-2-en-1-ol	t	t	0.1	t	t
1132	cis-limonene oxide	t	t	t	t	t
1136	trans-sabinol	t	t	0.1	t	t
1165	borneol	t	0.3	0.1	0.3	0.3
1174	terpinen-4-ol	0.4	0.3	0.4	0.4	0.4
1176	m-cymen-8-ol	0.1	0.2	0.1	0.2	0.1
1179	p-cymen-8-ol	0.2	0.2	0.2	0.2	0.3
1186	α-terpineol	t	0.2	0.1	0.1	t
1195	myrtenol	t	t	t	t	t
1204	verbenone	t	t	t	0.2	t
1232	thymol, methyl ether	0.3	0.4	0.6	0.5	0.7
1253	trans-sabinyl acetate	t	0.1	0.2	0.2	t
1254	linalool acetate	t	0.2	t	t	t
1284	**bornyl acetate**	**1.6**	**8.9**	**2.8**	**3.0**	**4.0**
1293	2-undecanone	0.3	0.2	0.3	0.2	t
1315	(2E,4E)-decadienal	t	t	t	0.1	t
1345	α-terpinyl acetate	2.2	1.3	2.1	1.3	1.2
1389	β-elemene	0.2	0.2	0.2	0.3	0.1
1417	**(E)-caryophyllene**	**5.1**	**6.8**	**4.2**	**4.2**	**6.1**
1454	α-humulene	0.8	1.1	0.7	0.6	0.9
1454	(E)-β-farnesene	0.2	0.3	0.2	t	t
1478	γ-muurolene	0.2	0.1	0.2	0.2	t
1480	**germacrene D**	**2.9**	**0.4**	**1.3**	**1.5**	**2.5**
1500	bicyclogermacrene	1.3	0.7	0.7	2.1	0.8
1500	α-muurolene	0.3	0.2	0.3	0.3	0.3
1508	germacrene A	0.3	0.2	0.3	0.7	0.2
1513	γ-cadinene	0.6	0.3	0.4	1.0	0.4
1522	δ-cadinene	1.3	1.0	1.3	1.3	1.0
1537	α-cadinene	t	t	0.1	t	t
1561	(E)-nerolidol	t	0.1	0.2	t	t
1574	**germacrene-D-4-ol**	**2.4**	**1.3**	**2.4**	**3.3**	**1.2**
1583	caryophyllene oxide	0.3	0.3	0.2	0.9	0.4
1638	epi-α-cadinol	0.5	0.3	0.9	0.6	0.3
1640	epi-α-muurolol	0.4	0.3	0.2	0.5	0.3
1644	α-muurolol	t	0.1	0.2	0.2	t
1652	α-cadinol	1.2	0.8	1.2	1.6	0.9
1710	pentadecanal	0.1	0.4	0.1	t	0.3
1880	(3Z)-hexenyl cinnamate	0.5	0.7	0.4	0.1	t
1943	iso-cembrene	t	t	0.2	t	0.4
1987	manool oxide	0.2	0.2	0.2	0.2	0.4
2056	abietatriene	t	t	t	0.3	t
2087	abietadiene	t	t	0.1	0.1	t
2105	iso-abienol	t	0.5	0.2	t	t
2149	abienol	t	t	t	t	t
2153	abieta-(8(14,13j(15)-diene	t	t	t	t	t

KI	compound	*15495*	*15496*	*15497*	*15498*	*15499*
2243	**palustral (8,13-abietadien-18-al**	**0.6**	**1.2**	**0.6**	**1.5**	**0.7**
2274	dehydro abietal	t	0.1	t	0.3	t
2313	abietal	t	0.1	t	0.1	t